

[RAPID COMMUNICATION]

Differential Expression of *Xenopus* BMPs in Early Embryos and Tissues

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ABSTRACT—Expression levels of mRNA for *Xenopus* bone morphogenetic proteins (BMPs) in early embryos and adult organs were examined. Reverse transcription-polymerase chain reaction (RT-PCR) that used specific primers for each BMP subtype revealed that mRNAs for BMP-2 and BMP-4 are distributed in a wide variety of adult tissues including lung, heart, and kidney. Unexpectedly, distribution of mRNA for BMP-7 was found to be limited to ovary and early embryos. The result suggests that *Xenopus* BMP-7 may have a specific role in ovary and early embryos.

INTRODUCTION

A growing body of evidence suggest that peptide growth factors play essential roles in animal development [4]. We have previously isolated genes encoding proteins related to activin, a member of transforming growth factor- β (TGF- β) family proteins from *Xenopus laevis* [12]. Among several activin related genes, one gene was found to code an extremely similar protein to mammalian bone morphogenetic protein-2 (BMP-2) [14], which was originally discovered for its ability to induce bone and cartilage formation *in vivo*. Subsequently, we have cloned cDNAs for BMP-2, -4 and -7 from *Xenopus* oocyte cDNA library using BMP-2 gene as a probe [8]. DNA sequence analysis has shown that their primary structures are highly conserved between amphibian and mammalian.

In the present study, ontogeny and tissue distribution of these *Xenopus laevis* BMPs were examined by reverse transcription PCR [6] that is a rather sensitive method to detect mRNAs from minute embryonic tissues.

MATERIALS AND METHODS

Xenopus embryos and tissues

Xenopus laevis embryos were obtained by artificial fertilization. The embryos were staged according to Nieuwkoop and Faber [7]. Adult organs are surgically removed from anesthetized male and female *Xenopus laevis*. All embryos and tissues were immediately frozen in liquid nitrogen and kept at -80°C until use.

Reverse transcription PCR

Total RNA was purified from embryos and adult tissues by acid guanidinium thiocyanate-phenol-chloroform extraction (AGPC) method [2] and guanidinium-CsCl method [1], respectively. RNAs from adult tissues were from mixture of several individuals. cDNA was synthesized from total RNA as follows. A 20 μl of reverse transcription mixture containing 500 ng of total RNA, 1 $\times$ RT buffer (50 mM Tris-HCl, pH 8.3/75 mM KCl/3 mM MgCl₂), 100 pmol of random hexamer primers, 0.5 mM of each dNTP, 10 mM DTT, 20 U of RNasin (Promega), and 100 U of MMLV reverse transcriptase (BRL) was incubated at 37 $^{\circ}\text{C}$ for 60

min, and heated to 95°C for 10 min. PCR was performed at a final concentration of 1×PCR buffer (10 mM Tris-HCl, pH 8.8/50 mM KCl/1.5 mM MgCl₂/0.1% Triton X-100)/0.2 mM each of dNTP/5 μCi of [α -³²P]dCTP (3000 Ci/mmol)/5 pmol of each primer/0.225 U of Taq DNA polymerase (Wako Chemical, Japan) in a total volume of 10 μl. The mixture was overlaid with mineral oil and then amplified with a thermal cycler (Perkin-Elmer/Cetus). The amplification profile involved denaturation at 95°C for 30 sec, primer annealing at 58°C for 30 sec, and extension at 72°C for 1 min. A 4 μl of the PCR sample was electrophoresed on 5% polyacrylamide gel and the PCR fragment was visualized by autoradiography. Appropriate bands were cut out from the dried gel and radioactivity was determined by Cerenkov counting. For quantitative analysis, optimal cycle number for each BMP was determined. Cycle of 22 was chosen for all BMPs because it is in the range of exponential increase of PCR products depending on cycle numbers and resulting radioactivities of PCR products are relative to RNA amounts under the amplification condition. Primers used in PCR were as follows: BMP-2 (5'-CAATGGTCGCTGGGATCCAC-3' and 5'-TCAACAACATTTTTGCCAGGCGT-3'), BMP-4 (5'-CATCATGATTCTGGTAACCGA-3' and 5'-CTCCATGCTGATATCGTGCAG-3'), BMP-7 (5'-AAGGCCACGAACGCAGA-3' and 5'-TGTCAGTTCATCTCCAAGT-3'), EF-1 α (5'-CCTGAATCACCCAGGCCAGATTGGTG-3' and 5'-GAGGGTAGTCTGAGAAGCTCTCCACG-3').

RESULTS AND DISCUSSION

Newly established RT-PCR method enabled us to detect mRNAs in small preparations of embryos and adult tissues compared to conventional methods such as Northern blot analysis and RNase protection assay. In addition, the method has minimized the time required for the assay. Presence of BMP mRNAs in developing embryos were demonstrated by this method using respective specific primers. Figure 1(a) schematically represents the precursor structures of BMPs and the regions chosen for designing oligonucleotide

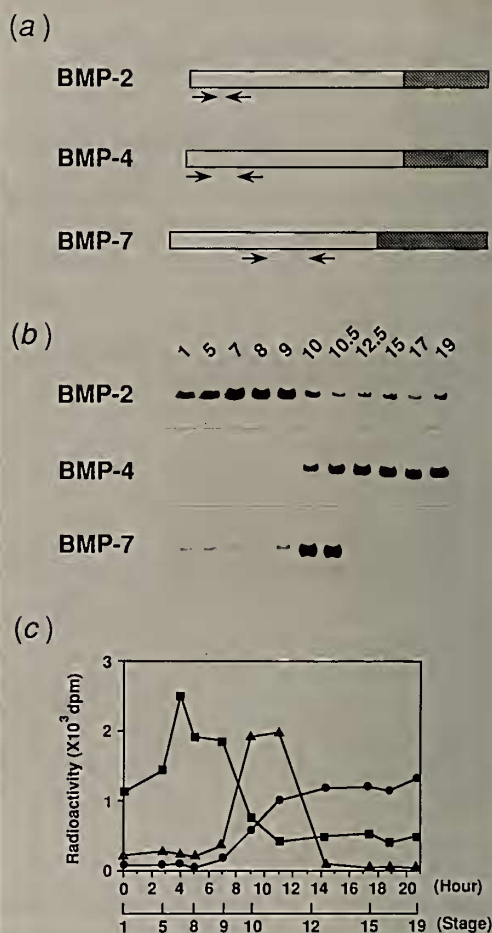


FIG. 1. Levels of BMP mRNAs during early *Xenopus* embryogenesis. (a) Primer positions used for RT-PCR. Each primer set was specifically designed to detect each BMP mRNAs and denoted by arrows. The propeptide structures that include mature region (shaded bars) derived from the full-length cDNAs are represented by bars. (b) Total RNA from staged embryos was subjected to RT-PCR using specific primers for each BMP mRNA. *Xenopus* embryos were cultured in 0.1× Steinberg's solution at 23°C. Developmental stages are shown to the top of the figure. (c) Schematic representation of (b). Radioactivity of appropriate band was measured by Cerenkov counting and plotted against hours after fertilization (BMP-2, squares; BMP-4, circles, BMP-7, triangles).

primers. Specific nucleotide sequences were chosen for each BMP to prevent the primers from cross-hybridization. Figure 1(b) shows the accu-

mulation of mRNA for *Xenopus* BMP-2, -4 and -7 during early embryogenesis. The result shows that all BMP mRNAs are maternally present and that the levels of the BMP mRNAs change drastically during development (BMP-4 transcript in early embryos before stage 9 becomes detectable by longer exposure). It should be noted that genes for BMPs are differentially regulated in early development although their primary structures are closely related to each other. Especially, homology of amino acid sequence of *Xenopus* BMP-2 and BMP-4 are 82% in their predicted mature proteins. Independent gene regulation of BMP genes in early embryos implies that they have distinct functions in embryogenesis. The result is very similar to that obtained by Northern blot analysis of poly(A)⁺ RNA purified from staged early embryos. This method, however, provided a sharper image of accumulation and decline of the mRNAs by plotting radioactivities of the PCR products (Figure 1(c)). Existence of BMP-2 [13] and BMP-4 [9] in embryo was also confirmed by Western blot analysis that used specific antibodies against their respective sequences.

Tissue distribution of BMP mRNAs was also examined. RNA was purified from adult tissues

and subjected to RT-PCR assay for BMP mRNAs. As shown in Figure 2, distributions of mRNAs for BMP-2 and -4 are very similar to each other and the transcripts were detected in a wide variety of tissues of not only mesoderm-origin but also ectoderm-origin. The result is in a good agreement with a previous observation on mammalian BMP-2 and -4 [15]. Interestingly, however, mRNA for *Xenopus* BMP-7 was detected only in ovary. In addition to the presence of MBP-7 mRNA in early embryo before neurula, this observation strongly suggests that BMP-7 has embryo-specific role(s) in *Xenopus* which is as yet unknown. It remains to be examined whether localization of mammalian counter part of *Xenopus* BMP-7 transcript is also limited to ovary and early embryos. It is known that a heterodimer of BMP-2 and -7 (OP-1) subunits is present in mammalian bone extracts [11]. Therefore, BMP-7 may modulate specificity and potency of BMP activities in ovary and early embryos by forming a heterodimer with another BMP peptide.

BMPs are structurally related to gene product of *Drosophila dppc* [10] which is a locus responsible for dorso-ventral specification and formation of imaginal disks in *Drosophila* embryo. Moreover,

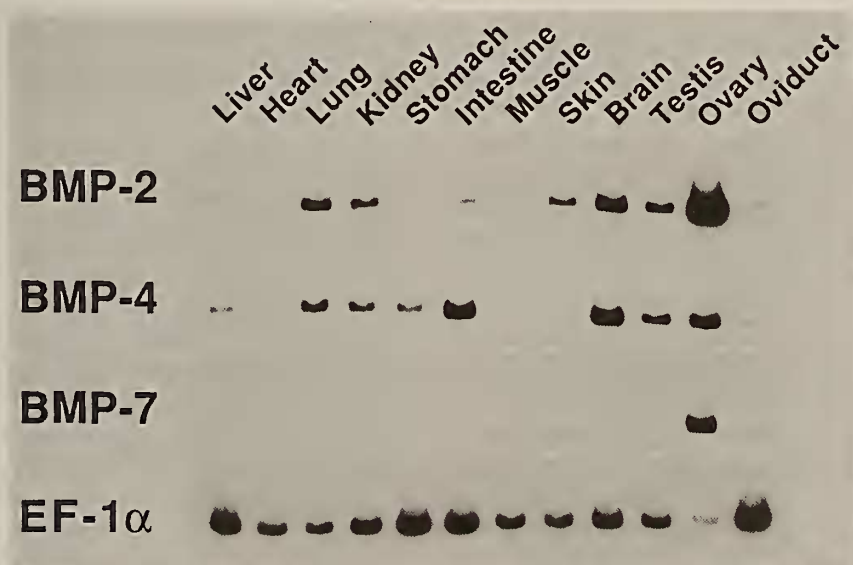


FIG. 2. Distribution of BMP mRNAs in adult *Xenopus* tissues. Total RNA (500 ng) from adult *Xenopus* tissues was subjected to RT-PCR using specific primers for each BMP mRNA and EF-1α mRNA. EF-1α was used to confirm both RNA quality and quantity. RT-PCR for EF-1α mRNA was performed at 19 cycles.

it has been proposed that *Xenopus* BMP-4 may function as a posterior-ventralizing factor in *Xenopus* [3, 5]. The presence of BMP-7 mRNA in "organizer field", dorsal lip of early gastrula (Dr. K. Cho, personal communication) suggests that *Xenopus* BMP-7 whose structure is similar to BMP-4 may also be an essential factor in pattern formation of amphibian development.

ACKNOWLEDGEMENTS

We wish to thank Dr. R. Makino for technical advice on RT-PCR. This study was supported in part by Grant-in-Aid for Scientific Research (No. 02558017) from Ministry of Education, Science and Culture, Japan and grants from Chichibu Cement Co. and Kowa Foundation for Promotion of Life Sciences.

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